भारतीय मानक
Indian Standard

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वर्मीकम्पोस्ट — विशिष्टि

Vermicompost — Specification

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Soil Quality and Fertilizers Sectional Committee, FAD 07

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Soil Quality and Fertilizers Sectional Committee had been approved by the Food and Agriculture Division Council.

Vermicompost, also called worm castings, worm humus or worm manure, is the end-product of the breakdown of organic matter by various types of worms. It is an odourless, clean, organic material containing adequate quantities of N, P, K and several micronutrients essential for plant growth.

Vermicompost is a preferred nutrient source for organic farming. Containing water-soluble nutrients, vermicompost is an excellent, nutrient-rich organic fertilizer and soil conditioner which is eco-friendly, non-toxic, consumes low energy input for composting and is a recycled biological product. These castings have been shown to contain reduced levels of contaminants and a higher saturation of nutrients than do organic materials before vermicomposting.

Vermicompost is rich in all essential plant nutrients. Provides excellent effect on overall plant growth, encourages the growth of new shoots/leaves and improves the quality and shelf life of the produce.

In the preparation of this standard, due consideration has been given to the *Municipal Solid Wastes (Management and Handling) Rules,* 2000, *Fertilizer (Control) Order*, 1957, *Standards of Weights and Measures Act*, 1977 and the *Essential Commodities Act*, 1955 of Government of India. However, this standard is subject to restrictions imposed under these, wherever applicable.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

VERMICOMPOST — SPECIFICATION

1 SCOPE

This standard prescribes the requirements, methods of sampling and tests for Vermicompost, manure grade.

2 REFERENCES

The following standards contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

IS No.	*Title*
460	Specification for test sieves:
(Part 1) : 1985	Wire cloth test sieves (*third revision*)
(Part 2) : 1985	Perforated plate test sieves (*third revision*)

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1 Vermicompost — Vermicompost is an organic manure (bio-fertilizer) produced as the vermicast using various worms, usually red wigglers, white worms, and other earthworms feeding on biological waste material; plant residues to create a heterogeneous mixture of decomposing vegetable or food waste, bedding materials, and vermicast.

3.2 Compost — Substance made of one or more unprocessed waste material of biological nature (plant and animal) and may include unprocessed mineral material that has been altered through microbiological decomposition.

4 REQUIREMENTS

4.1 Description

The vermicompost shall be in powdered or granular form, dark brown to black in colour with no foul odour.

4.2 Particle Size

The particle size of vermicompost shall not be more than 4.0 mm, that is minimum 90 percent of vermicompost shall pass through 4.0 mm IS sieve [*see* IS 460 (Part 1 and Part 2)].

4.3 The product shall also conform to the requirements given in Table 1.

Table 1 Requirements for Vermicompost

(*Clause* 4.3)

Sl No.	Characteristic	Requirement	Method of Test, Ref to
(1)	(2)	(3)	(4)
i)	*p*H	6.5-7.5	Annex A
ii)	Moisture, per cent by weight	15.0-25.0	Annex B
iii)	Bulk density (g/cm^3)	0.7 -0.9	Annex C
iv)	Conductivity (as dSm^{-1}), *Max*	4.0	Annex D
v)	Total organic carbon, per cent by weight, *Min*	18.0	Annex E
vi)	Total Nitrogen (as N), per cent by weight, *Min*	1.0	Annex F
vii)	C:N ratio, *Max*	20:1	Annex G
viii)	Total phosphorus(as P_2O_5), percent by mass of total dry mass, *Min*	0.8	Annex H
ix)	Total potassium (as K_2O), per cent by weight, *Min*	0.8	Annex J
x)	Cadmium (as Cd), mg/kg on dry mass basis, *Max*	5.0	Annex K
xi)	Copper (as Cu), mg/kg on dry mass basis, *Max*	300.00	Annex K
xii)	Chromium (as Cr), mg/kg on dry mass basis, *Max*	50.00	Annex K
xiii)	Lead (as Pb), mg/kg on dry mass basis, *Max*	100.00	Annex K
xiv)	Nickel (as Ni), mg/kg on dry weight basis, *Max*	50.00	Annex K
xv)	Mercury (as Hg), mg/kg on dry mass basis, *Max*	0.15	Annex L
xvi)	Arsenic (as As_2O_3), mg/kg on dry mass basis, *Max*	10.00	Annex M
xvii)	Pathogens	Absent	Annex N

5 PACKING AND MARKING

5.1 Packing

The material shall be packed in the manner as agreed to between the purchaser and the supplier. Each bag shall be securely closed.

5.2 Marking

Each bag shall bear legibly the following information:

a) Name and type of the material;
b) Name of the manufacturer;
c) Indication of the source of manufacture;
d) Percent by mass of total nitrogen to total dry mass;
e) Percent by mass of total phosphorous to total dry mass;
f) Percent by mass of total potassium to total dry mass;
g) Percent by mass of total organic carbon to total dry mass;
h) C:N ratio;
j) Gross and net quantity in kg;
k) Batch number, in code or otherwise, to enable the lot of manufacture to be traced back from records; and
m) Any other information as required under FCO.

5.2.1 *BIS Certification Marking*

The container may also be marked with the Standards Mark.

5.2.1.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standard Act*, 2016 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6 SAMPLING

6.1 General Requirements of Sampling

In drawing samples, the following measures and precautions should be observed:

a) Samples shall not be taken at a place exposed to rain /sun.
b) The sampling instruments shall be clean and dry when used.
c) The material being sampled, the sampling instruments and the bags of samples should be free from any adventitious contaminations.
d) To draw a representative sample, the contents of each bag selected for sampling should be mixed as thoroughly as possible by suitable means.
e) The sample should be kept in suitable, clean dry and airtight glass or screwed hard polythene bottle of about 400gm capacity or in a thick-gauged polythene bag. This should be put in a cloth bag, which may be sealed with the Inspector's seal after putting inside the detailed description as specified in Form 'P'. Identifiable details may also be put on the cloth bag like sample No./Code No. or any other details which enables its identification.
f) Each sample bag should be sealed air tight after filling and marked with details of sample and type of fertilizer and the name of Inspector who has collected sample.

6.2 Sampling from Bagged Material

6.2.1 *Scale of Sampling*

a) *Lot (for manufacturers/importers)* — All bags in a single consignment of the material of the same grade and type drawn from a single batch of the manufacturer/importer shall constitute a lot. If a consignment is declared to consist of different batches of manufacturer/import, all the bags of each batch shall constitute a separate lot. In the case of a consignment drawn from a continuous process, 2 000 bags (or 100 tonnes) of the material shall constitute a lot.
b) *Lot (for dealer)* — The lot is an identifiable quantity of same grade and type of fertilizer stored at an identifiable place subject to a maximum limit of 100 tonnes. The inspector based on visible appearance of bags, their packing and storage conditions shall identify the lot. The stock of less than 100 tonnes with a dealer may also constitute one or more lots, if the material (fertilizer) of different sources and brand is available in such quantities.
c) *Selection of Bags for Sampling* — The number of bags to be chosen from a lot shall depend upon the size of the lot as given in Table 2.

Table 2 Selection of Bags for Sampling
[*Clauses* 5.2.1 to 6.2.1 (c)]

Lot Size (No. of Bags) (*N*) (1)	No. of Bags to be Selected for Sampling (*n*) (2)
Up to 10	1
11 to 100	2
101 to 200	3
201 to 400	4
401 to 600	5
601 to 800	6
801 to 1 000	7
1 001 to 1 300	8
1 301 to 1 600	9
1 601 to 2 000	10

d) All the bags of a lot should be arranged in a systematic manner. Start counting from any bag randomly; go on counting as 1, 2, 3, ………….up to *R* and so on. *R* being equal to the integral of *N/n*. Thus every 4th bag counted shall be withdrawn and all bags shall constitute the sample bags from where the sample is to be drawn for preparing a composite sample.

6.2.2 *Sampling from Big Godowns/High Stackings*

If the procedure given in **6.2.1** (c) is not possible to be adopted, the sample should be drawn from the randomly selected fertilizer bags from different layers, from top and from all open sides in a zig zag fashion.

6.2.3 *Sampling from Small Godowns*

All the fertilizer bags of the same grade and type of each manufacturer though received on different dates shall be segregated and properly stacked. All bags of same grade and type of fertilizer manufactured by a particular manufacturing unit may be considered as one lot based on their physical conditions and the sample shall be drawn as per procedure laid down in **6.2.1** (c) and (d).

6.2.4 *Sampling from damaged stocks*

a) In case of torn or lumpy bags, damaged fertilizer bags or sweepings, the stock should be arranged according to identifiable lots. From each lot the number of bags shall be selected as per procedure **6.2.1** (c). If the bags allow the use of sampling probe conveniently, the samples should be drawn from sampling probe.

b) In case it is not possible to use the sampling probe, the bags may be opened and fertilizer material mixed together uniformly by hammering the big lumps or putting pressure, if required, and then samples drawn by using suitable sample device.

6.3 Sampling Probe

a) An appropriate sampling instrument to be used by the Inspector for collection of a representative sample is called sampling probe. The probe may comprise of a slotted single tube with solid cone tip made of stainless steel or brass. The length of the probe may be approximately 60 cm to 65 cm and the diameter of the tube may be approximately 1.5 cm and the slot width may be 1.2 cm to 1.3 cm. The probe may be used if the physical condition of the fertilizers and the packing material permits its use.

b) In case of high-density polythene packing and also when the fertilizer material is not in free flowing condition, the use of sampling probe may not be possible. In such case, selected bags for drawing samples may be opened and the fertilizers may be taken out of the bags and spread on a clean surface and samples drawn with the help of a suitable sampling device, which may be made of stainless steel or brass cup.

6.4 Drawl of Samples from Bags

a) Drawl of sample and preparation of composite samples. Draw, with an appropriate sampling instrument, (sampling probe) small portions of the material from the selected bags as per procedure in **6.2.1**(b), **6.2.2**, **6.2.3** and **6.2.4** (a). The sampling probe shall be inserted in the bag from one corner to another diagonally and when filled with fertilizer, the probe is withdrawn and fertilizer is emptied in a container/ or on polythene sheet/ or on a clean hard surface and made into one composite sample.

b) If the bags do not permit the use of sampling probe, empty the contents of the bags on a level, clean and hard surface and draw a composite sample by the process of quartering as described under **6.6**.

6.5 Quantity of One Sample

One sample of organic fertilizer shall have approximate 400 g weight.

6.6 Preparation of Composite Sample

If the composite sample collected from the different selected bags is larger than required weight, its size shall be reduced by method of quartering as detailed below:

> Spread the composite sample on a level, clean, hard surface, flatten it out and divide it into four equal parts. Remove any diagonally opposite parts. Mix the two remaining parts together to form a cone, flatten out the cone and repeat the operation of quartering till a composite sample of required weight is obtained.

6.7 Preparation of Test Sample and Reference Sample

a) The composite sample obtained above shall be spread out on a clean, hard surface and divided into three approximately equal portions each of the weight as specified in **6.5**. Each of these samples shall constitute the test sample.

b) Each test sample shall be immediately transferred to a suitable container as defined under **6.1**(e). The slip with detailed description may be put inside the sample bag. Each bag shall also be properly labeled as mentioned in **6.1**(f).

c) Each test sample container shall then be sealed with seals of the inspector, if possible, seal of the manufacturer/ importer/ dealer or purchaser as the case may be also be affixed.

d) Out of the three samples collected, one sample so sealed shall be sent to the Incharge of the laboratory notified by the state Government under clause 29 or National/Regional Centre of Organic Farming, Ghaziabad, Bangalore, Bhubaneshwar, Hisar, Imphal, Jabalpur or Nagpur for analysis. Another sample shall be given to the manufacturer or importer or dealer or the purchaser, as the case may be. The third sample shall be sent by the Inspector to his next higher authority for keeping in safe custody. Any of the latter two samples may be sent for referee analysis as provided for under sub-clause (2) of clause 29B of *Fertilizer (Control) Order* 1985.

ANNEX A

[*Table* 1, *Sl No.* (i), *col* 4]

ESTIMATION OF *p*H

A-1 REAGENT

Distilled water.

A-2 APPARATUS

Rotary shaker, Whatman No. 1 or equivalent filter paper, Buchner funnel fitted with vacuum pump, *p*H meter.

A-3 PROCEDURE

Make 25 g of compost into a suspension in 50 ml of distilled water and shake on a rotary shaker for 2 h. Filter through Whatman No. 1 or equivalent filter paper under vacuum using a Buchner funnel. Determine *p*H of the filtrate by *p*H meter.

ANNEX B

[*Table* 1, *Sl No.* (ii), *col* 4]

ESTIMATION OF MOISTURE

B-1 APPARATUS

Petri dish, Hot air oven, desiccator, weighing balance.

B-2 PROCEDURE

Weigh to the nearest mg about 5 g of the prepared sample in a weighed clean, dry Petri dish. Heat it in an oven for about 5 h at 65±1°C to constant weight. Cool in a desiccator and weigh. Reportthe percentage loss in weight as moisture content.

B-3 CALCULATION

$$\text{Moisture percent by mass} = \frac{100\,(B-C)}{B-A}$$

where

A = mass of the Petri dish;

B = mass of the Petri dish with sample before drying; and

C = mass of the Petri dish with sample after drying.

ANNEX C

[*Table* 1, *Sl No.* (iii), *col* 4]

ESTIMATION OF BULK DENSITY

C-1 APPARATUS

Weighing balance, measuring cylinder (100 ml), Rubber pad (0.093 m^2; 2.57 cm thickness), Hot air oven.

C-2 PROCEDURE

Weigh a dry 100 ml cylinder (W_1). Fill the cylinder with the sample up to the 100 ml mark. Note the volume (V_1). Weigh the cylinder along with the sample (W_2). Tap the cylinder for 2 min for compacting the sample. Measure the compact volume (V_2).

C-3 CALCULATION

$$\text{Bulk density (g/cm}^3) = \frac{W_2 - W_1}{V_1 - V_2}$$

where

W_1 = weight of the dry 100 ml cylinder in g;

W_2 = weight of the dry 100 ml cylinder with sample, in g;

V_1 = initial volume up to which the sample was filled in the cylinder in ml = 100 ml; and

V_2 = final volume up to which the sample is present in the cylinder after compaction in ml.

ANNEX D

[*Table* 1, *Sl No.*(iv), *col* 4]

ESTIMATION OF CONDUCTIVITY

D-1 REAGENTS

Potassium Chloride (AR grade), distilled water.

D-2 APPARATUS

Conductivity meter (with temperature compensation system), 4 mm sieve, flask (250 ml), funnel (outer diameter = 75 mm), beaker (100 ml), analytical balance, filter paper.

D-3 PROCEDURE

Pass fresh sample of organic fertilizer through a 2-4 mm sieve. Take 20 g of the sample and add 100 ml of distilled water to it to give a ratio of 1:5. Stir for an hour at regular intervals. Calibrate the conductivity meter by using 0.01 M potassium chloride solution. Measure the conductivity of the unfiltered compost suspension.

D-4 CALCULATION

Express the results as millimho's or dsm^{-1} at 25°C specifying the dilution of the organic fertilizer suspension namely, 1:5 organic fertilizer suspensions.

ANNEX E

[*Table* 1, *Sl No.* (v), *col* 4]

ESTIMATION OF TOTAL ORGANIC CARBON

E-1 APPARATUS

Silica/Platinum crucible (25 g) cap, muffle furnace, analytical balance, hot air oven, desiccator.

E-2 PROCEDURE

Accurately weigh 10 g oven dried sample (105°C for 6 h) in to a pre-weighed crucible and ignite the material in a Muffle furnace at 650-700°C for 6-8 h. Cool to room temperature and keep in desiccator for 12 h.Weigh the contents with crucible.

E-3 CALCULATION

Calculate the total organic carbon by the following formulae:

$$\text{Total organic matter (percent by mass of total dry mass)} = \frac{W_1 - W_2 \times 100}{W}$$

where

W = weight of the sample = 10 g,

W_1 = initial weight of the silica crucible with the sample, and

W_2 = final weight of the silica crucible with the sample after ignition.

Total organic carbon
(percent by mass of total dry mass)

$$= \frac{\text{Total organic matter (pecent by mass of total mass)}}{1.724}$$

ANNEX F

[*Table* 1, *Sl No.* (vi), *col* 4]

ESTIMATION OF TOTAL NITROGEN

F-1 APPARATUS

F-1.1 Suitable Kjeldahl assembly consisting of 500-800 ml round bottom, digestion flask and Kjeldahl distillation assembly consisting of 500-800 ml distillation flask, splash head tube and condenser, all with appropriate glass joints. The length of the condenser's delivery tube should be long enough to keep immersed in a flask for ammonia absorption.

F-1.2 Kjeldahl digestion unit with heating control, suitable for 500-800 ml flasks.

F-2 REAGENTS

F-2.1 Sulphuric Acid — 93-98 percent H_2SO_4, N-free.

F-2.2 Salicylic Acid, reagent grade, N-free.

F-2.3 Sodium Thiosulphate ($Na_2S_2O_3$ $5H_2O$), reagent grade.

F-2.4 Zinc Dust — impalpable powder.

F-2.5 Copper Sulphate

F-2.6 Potassium or Sodium Sulphate

F-2.7 45 percent NaOH Solution — Dissolve 450 g of sodium hydroxide pellets in distilled water and make up the volume to 1 000 ml.

F-2.8 Methyl Red Indicator — Dissolve 1g methyl red in 200 ml alcohol.

F-2.9 Hydrochloric or Sulphuric Acid Standard Solution — 0.1 N or as per requirement.

F-2.10 Sodium Hydroxide Standard Solution– 0.1 N or as per requirement.

F-3 PROCEDURE

Place weighed sample (0.7-2.2g) in digestion flask. Add 40 ml H_2SO_4 containing 2 g salicylic acid. Shake until thoroughly mixed and let stand, with occasional shaking, 30 min or more. Then add (a) 5 g $Na_2S_2O_3.5H_2O$ or (b) 2 g zinc dust (as impalpable powder not granulated zinc or filing). Shake the flask and let it stand for 5 min then heat over low flame until frothing ceases.

Turn off heat, add 0.7 g copper sulphate, 15 g powdered K_2SO_4 (or anhydrous Na_2SO_4)and boil briskly until solution clears, continue boiling for another at least 2 h. Remove from burner and cool, add 200 ml of water and swirl the flask to dissolve all the contents. Transfer to 500 ml volumetric flask, giving several washings with water to the digestion flask. Make up the volume to 500 ml.

Take 25 ml aliquot in the distillation flask, add 300 ml water and a pinch of zinc dust. Take 20 ml of standard acid solution in the receiving conical flask, add 4 drops of methyl red indicator and keep the flask at the lower end of the condenser in such a way that the lower tip of the condenser is fully immersed in acid solution.

Add 30 ml of 45 percent NaOH to the distillation flask, gently so that the contents do not mix. Immediately connect the flask to distillation assembly and swirl to mix the contents. Heat until all the ammonia is distilled (at least 150 ml distillate). Remove from receiving flask. Rinse outlet tube into receiving flask with a small amount of distilled water.

Titrate the contents in the receiver conical flask with standard NaOH solution. Determine blank on reagents using same quantity of standard acid in receiving conical flask.

F-4 CALCULATION

$$\text{Total nitrogen (as N), percent by mass of total dry mass} = \frac{1.401\,(V_1N_1 - V_2N_2) - (V_3N_1 - V_4N_2) \times df}{W}$$

where

V_1 = volume in ml of standard acid taken in receiver flask for sample;

V_2 = volume in ml of standard NaOH used in titrating standard acid in receiver flask after distillation of test sample;

V_3 = volume in ml of standard acid taken in receiver flask for blank;

V_4 = volume in ml of standard NaOH used in titrating standard acid in receiver flask after distillation in blank;

N_1 = normality of standard acid;

N_2 = normality of standard NaOH;

W = weight in gm of sample taken; and

df = dilution factor of sample.

ANNEX G

[*Table* 1, *Sl No.* (vii), *col* 4]

CALCULATION OF C : N RATIO

G-1 The C:N ratio is calculated using total organic carbon, percent by mass of total dry mass determined at **E-3** and total nitrogen (as N), percent by mass of total dry mass determined at **F-4**.

G-2 CALCULATION

C : N ratio $= A : B$

where

A = total organic carbon, percent by mass of total dry mass determined at **E-3**; and

B = total nitrogen (as N), percent by mass of total dry mass determined at **F-4**.

ANNEX H

[*Table* 1, *Sl No.* (viii), *col* 4]

ESTIMATION OF PHOSPHOROUS

H-1 PREPARATION OF SAMPLE

H-1.1 Reagent

25 percent HCl, distilled water.

H-1.2 Apparatus

Silica crucible (50 g) capacity, desiccator, beaker (100 ml), Whatman No.1 filter paper, hot plate, volumetric flask (100 ml).

H-1.3 Procedure

Accurately weigh 10 g oven dried sample in silica crucible 50 g capacity and ignite it to 650– 700°C for 6-8 h to obtain ash. Cool and keep in a desiccator.

Transfer the contents to a 100 ml beaker. Add 30 ml 25 percent HCl. Wash the crucible with 10 ml 25 percent HCl twice and transfer the contents to beaker. Heat over hot plate for 10-15 min. Keep for 4 h. Filter through Whatman No.1 filter paper. Wash with distilled water 4-5 times (till acid free). Make up the volume of filtrate to 100 ml in a volumetric flask. Estimate total phosphorus by gravimetric quinoline method as describe in **H-2**.

H-2 GRAVIMETRIC QUINOLINE METHOD FOR DETERMINATION OF TOTAL PHOSPHORUS

H-2.1 Reagents

H-2.1.1 *Citric Molybdic Acid Reagent* — Dissolve 54 g, 100 percent molybdic anhydride $(Mo)_3$ and 12 g NaOH with stirring in 400 ml hot water and cool. Dissolve 60 g citric acid in mixture of 140 ml HCl and 300 ml water and cool. Gradually add molybdic solution to citric acid solution with stirring. Cool, filter and dilute to 1 litre (solution may be green or blue colour depending on exposure to light). If necessary add 0.5 percent $KBrO_3$ solutiondrop by drop until green colour becomes pale. Store in dark in polyethylene bottle.

H-2.1.2 *Quinoline Solution* — Dissolve 50 ml synthetic quinoline with stirring in mixture of 60 ml HCl and 300 ml water. Cool dilute to 1 litre and filter. Store in polyethylene bottle.

H-2.1.3 *Quimociac Reagent* — Dissolve 70 g of sodium molybdate dehydrate in 150 ml water. Dissolve 60 g citric acid in mixture of 85 ml HNO_3 and 150 ml water and cool. Gradually add molybdate solution to citric acid-nitric acid mixture with stirring. Dissolve 5 ml synthetic quinoline in mixture of 35 ml HNO_3 and 100 ml water. Gradually add this solution to molybdate –citric-nitric acid solution mix and let it stand for 24 h. Filter, add 280 ml acetone, dilute to 1 litre with water and mix well. Store in polyethylene bottle.

H-2.2 Apparatus

Erlenmeyer flask, pipette, watch glass, hot plate, sintered glass gooch crucible Grade 4 (30 ml capacity), desiccator.

H-2.3 Procedure

Digest 1 g sample as described above and dilute to 200 ml. In 500 ml Erlenmeyer flask pipette aliquot containing not more than 25mg P_2O_5 dilute to approximately 100 ml with water. Proceed with one of the following method.

a) Add 30 ml citric-molybdic acid reagent and boil gently for 3 min (solution must be precipitate free at this stage). Remove from heat and swirl carefully. Immediately add from burette 10 ml quinoline solution with continuous swirling (add first 3-4 ml drop wise and remainder in steady stream).

Or

b) Add 50 ml quimociac reagent, cover with watch glass place on hot plate in well ventilated hood and boil for 1 min.

After treatment with (a) or (b) cool to room temperature, swirl carefully 3-4 time during cooling, filter through sintered glass Gooch crucible Grade 4 (30 ml capacity), previously dried at 250°C and weighed, and wash 5 times with 25 ml portion of water. Dry crucible and contents for 30 min at 250°C. Cool in desiccator to constant weight as quinolinephosphomolybdate $[(C_9H_7N)_3H_3PO_4.12MoO_3]$.

Estimate blank reading without sample.

Subtract blank weight. Multiply by 0.03207 to obtain weight of P_2O_5. Report as percent P_2O_5.

H-2.4 Calculation

Total phosphorus (as P_2O_5), percent by mass of total dry mass = $(A - B) \times 0.032\,07$

where

A = weight of quinoline phosphomoly bdate obtained by treatment of sample, in g; and

B = weight of quinolinephosphomolybdate in blank treatment in g.

ANNEX J

[*Table* 1, *Sl No.* (ix), *col* 4]

ESTIMATION OF POTASSIUM

J-1 PREPARATION OF STANDARD CURVE

J-1.1 Reagents

J.1.1.1 *Potassium chloride standard solution* — make a stock of 1 000 ppm potassium by dissolving 1.909 g of AR Grade potassium chloride (dried at 60°C for 1 h) in distilled water; and diluting up to 1 litre. Prepare 100 ppm standard by diluting 100 ml of 1 000 ppm stock solution to 1 litre with extracting solution.

J-1.2 Apparatus

Analytical balance, pipette, volumetric flasks (100 ml), flame photometer with K-filter.

J-1.3 Procedure

Pipette 0, 5, 10, 15 and 20 ml of 100 ppm solution in to 100 ml volumetric flasks and make up the volume up to the mark. The solutions contain 0, 5, 10, 15, and 20 ppm potassium respectively. Determine potassium by the flame photometer using K-filter after necessary setting and calibration of the instrument. Prepare the standard curve plotting the reading against the different concentration of the potassium.

J-1 ESTIMATION OF POTASSIUM CONTENT OF SAMPLE

J-1.1 Reagents

Concentrated hydrochloric acid, distilled water

J-1.2 Apparatus

Porcelain crucible, muffle furnace, volumetric flask (100 ml), flame photometer with K-filter

J-1.3 Procedure

Take 5 g sample in a porcelain crucible and ignite the material to ash at 650-700°C in a muffle furnace. Cool it and dissolve in 5ml concentrated hydrochloric acid. Transfer to a 250 ml beaker with several washing of distilled water and heat it. Again transfer it to a 100 ml volumetric flask and make up the volume. Filter the solution and dilute the filtrate with distilled water so that the concentration of K in the working solution remains in the range of 0 to 20 ppm, if required. Determine K by the flame photometer using K-filter after necessary setting and calibration of the instrument.

J-1.4 Calculation

Potassium (as K_2O), percent by mass of total dry mass $= R \times 20 \times$ diluting factor

where

R = ppm of K in the sample solution (obtained by extrapolating from standard curve).

ANNEX K

[*Table* 1, *Sl No.* (x) to (xiv), *col* 4]

ESTIMATION OF CADMIUM, COPPER, CHROMIUM, LEAD AND NICKEL

K-1 REAGENTS

K-1.1 Triacid Mixture — Mix 10 parts of Nitric acid (HNO_3). 1 part of sulphuric acid (H_2SO_4) and 4 parts of Perchloric acid ($HClO_3$)

K-1.2 Standard Cadmium Solution

K-1.3 Standard Copper Solution

K-1.4 Standard Chromium Solution

K-1.5 Standard Lead Solution

K-1.6 Standard Nickel Solution

K-1.7 Double Distilled Water

K-2 APPARATUS

Analytical balance, hot air oven, 0.2 mm sieve, conical flask (250 ml), glass funnel, hot plate, Whatman filter paper No.42, atomic absorption spectrophotometer, volumetric flasks (100 ml)

K-3 PROCEDURE

K-3.1 Processing of Sample

Take 5.0 g or suitable quantity of oven dried (105°C) sample thoroughly ground and sieved through 0.2 mm sieve in a conical flask. Add 30 ml triacid mixture, cover it up with a small glass funnel for refluxing. Digest the sample at 200°C on a hot plat till the volume is significantly reduced with a whitish residue. After cooling, filter the sample through Whatman filter paper No. 42. Make up the volume to 100 ml with double distilled water in a volumetric flask.

K-3.2 Preparation of Working Standards

Dilute 1, 2, 3 and 4 ml of standard 100 ppm cadmium, copper, chromium, lead and nickel standard solution with double distilled water in separate volumetric flasks and makeup the volumes to 100 ml to obtain standards having concentrations of 1, 2, 3 and 4 ppm.

K-3.3 Measurement

Estimate the metal concentrations of cadmium, copper, chromium, lead and nickel by flaming the standards and samples using atomic absorption spectrophotometer (AAS) as per the method given for instrument at recommended wavelength for each element. Run a blank following the same procedure.

K-3. Expression of Result

Express the metal concentration as mg/kg on oven dry mass basis.

ANNEX L

[*Table* 1, *Sl No.* (xv), *col* 4]

ESTIMATION OF MERCURY

L-1 REAGENTS

L-1.1 Conc Nitric Acid (HNO_3)

L-1.2 Conc Sulphuric Acid (H_2SO_4)

L-1.3 Potassium Persulphate (5 percent Solution) — Dissolve 50g of $K_2S_2O_8$ in 1 litre of distilled water.

L-1.4 Potassium Permanganate (5 percent Solution) — Dissolve 50 g of $KMnO_4$ in 1 litre of distilled water.

L-1.5 Hydroxylamine Sodium Chloride Solution — Dissolve 120 g of Hydroxylamine salt and 120 g of sodium chloride (NaCl) in 1 litre of distilled water.

L-1.6 Stannous Chloride (20 percent) — Dissolve 20 g of $SnCl_2$ in 100 ml distilled water.

L-1.7 Standard Mercury Solution

L-1.8 Double Distilled Water

L-2 APPARATUS

L-2.1 Water Bath

L-2.2 Flameless Atomic Absorption Spectrophotometer (AAS attached with vapour generation assembly) or cold vapour mercury analyzer.

L-2.3 BOD Bottle (300 ml)

L-2.4 Analytical Balance

L-2.5 Hot Air Oven

L-2.6 Volumetric Flask (100 ml)

L-3 PROCEDURE

L-3.1 Processing of Sample

Take 5 g (finely ground but not dried) sample in oven at temperature of 105°C for 8 h for moisture estimation. Take another 5 g sample (finely ground but not dried) in a BOD bottle, add to it 2.5 ml ofconcHNO_3, 5 ml of conc H_2SO_4 and 15 ml of 5 percent $KMnO_4$. After 15 min add 8ml of 5 percent $K_2S_2O_8$. Close the bottle with the lid and digest it on the water bath at 95° C for 2 h. After cooling to room temperature add 5 ml hydroxylamine sodium chloride solution.

L-3.2 Preparation of Standard

Prepare standards having concentrations of 0.05, 0.1 and 0.2 ppm by diluting 0.05, 0.1 and 0.2 ml of standard Arsenic solution with double distilled water in a volumetric flask and make up the volume to 100 ml.

L-3.3 Measurement

Reduction of digested sample is brought out with 5 ml of 20 percent $SnCl_2$ immediately before taking the readings. Estimate mercury concentration using a cold vapour mercury analyzer or Atomic Absorption Spectrophotometry as per the method given for instrument. Run a blank following the same procedure.

L-3.4 Expression of Results

Express the mercury concentration as mg/ kg on oven dry mass basis.

ANNEX M

[*Table* 1, *Sl No.* (xvi), *col* 4]

ESTIMATION OF ARSENIC

M-1 REAGENTS

Aquaregia (HNO_3 + HCl in a ratio of 1:3), conc hydrochloric acid, double distilled water, potassium iodide, standard arsenic solution

M-2 APPARATUS

Analytical balance, beaker, hot plate, Whatman No.1 filter paper, volumetric flask (100 ml), Atomic Absorption Spectrophotometer attached with vapour generation assembly.

M-3 PROCEDURE

M-3.1 Processing of Sample

Suspend 10g finely ground sample in 30 ml aquaregia (HNO_3 + HCl in a ratio of 1:3) in a beaker. Keep on hot plate till moist black residue is obtained (do not dry). Add 5 ml aquaregia and allow to dry on hot plate till residue is moist. Dissolve the residue in 30 ml concHCl and filter through Whatman No.1 filter paper in 100 ml volumetric flask. Wash filter paper 3-4 times with double distilled water. Make up the volume to 100 ml. Take 1 ml of this solution in 100 ml volumetric flask, add 5ml conc. hydrochloric acid and 2 g potassium iodide and make up the volume to 100 ml.

Prepare standards having concentrations of 0.05, 0.1 and 0.2 ppm by diluting 0.05, 0.1 and 0.2 ml of standard Arsenic solution with double distilled water in a volumetric flask by making up the volume to 100 ml.

M-3.2 Measurement

Estimate Arsenic using vapour generation assembly attached to Atomic Absorption Spectrophotometer as per the method given for instrument.

M-3.3 Expression of Result

Express the metal concentration as mg/kg on oven dry mass basis.

ANNEX N

[*Table* 1, *Sl No.* (xvii), *col* 4]

PATHOGENICITY TEST

N-1 MEDIA

N-1.1 Lactose Broth for Presumptive Test

Beef Extract	:	6.0g
Peptone	:	10.0g
Lactose	:	10.0g
Distilled Water	:	1 000 ml

N-1.2 Eosine Methylene Blue Agar Media (EMB Media) for Confirmative Test

Peptone	:	10.0g
Lactose	:	5.0g
Sucrose	:	5.0g
K_2HPO_4	:	2.0g
Eosine Y	:	0.4g
Methylene Blue	:	0.06g
Agar	:	15.0g
Distilled Water	:	1000ml

N-1.3 Nutrient Agar for Completed Test

Beef Extract	:	3.0g
Peptone	:	5.0g

N-1.4 Gram's Crystal Violet

N-1.5 Gram's Iodine

N-1.6 95 percent Alcohol

N-1.7 0.25 percent Gram's Safranin

N-2 APPARATUS

Culture Tubes, Durham Tubes, Bunsen Burner, Sterile Pipettes, Incubator, Autoclaves, Petri-Plates, Inoculation Loops, Glass slides, Paper towel, Burner, Microscope.

N-3 PROCEDURE

N-3.1 Presumptive Test

Prepare 12 tubes of lactose broth for each sample and close the tube with cotton plugs/caps and autoclave at 121°C for 20 min. Fill Durham tubes with sterilized distilled water and keep in beaker and autoclave at 121°C for 20 min. Suspend 30g of compost sample in 270 ml of sterile distilled water and serially dilute upto 10^{-4} dilution. Suspend 1 ml suspension from 10^{-1} to 10^{-4} in 3 tubes for each dilution. Insert distilled water filled Durham tube in inverted position in each tube and close the tube again. Inoculate tubes at 36°C for 24h in incubator.

Production of gas within 24h indicates the presence of coliforms in the sample, that is, the sample has to be tested further for pathogenicity through confirmative test. If no gas production is observed incubate the tubes at 36°C for further 24h. Production of gas within 48 h indicates doubtful test that is, the sample has to be tested further for pathogenicity through confirmative test. No gas production within 48h indicates negative test that is., sample passes the pathogenicity test. No further testing is required.

N-3.2 Confirmative Test

Prepare EMB agar plates. Inoculate plates with the help of inoculation loop with streaking of samples showing positive / doubtful tests in the presumptive test. Incubate plates at 30± 1°C for 12 h in incubator. Observe the colonies appearing on the plate. Coliforms produce small dark centred or nucleated colonies with metallic sheen. Non-coliforms usually produce large colonies that lack metallic sheen.

Absence of small dark centred or nucleated colonies with metallic sheen confirms the absence of coliforms in the sample ie., sample passes the pathogenicity test. No further testing is required. Presence of small dark centred or nucleated colonies with metallic sheen confirms the presence of coliforms in the sample that is, sample the sample has to be tested further for pathogenicity through completed test.

N-3.3 Completed Test

Pick up a single small dark centered or nucleated colony with metallic sheen from EMB agar plate. Inoculate it into lactose broth and streak on a nutrient agar slant. Incubate the slants.

Prepare the smears of the colonies in glass slides and heat-fix smears. Flood the slides with crystal violet for one minute. Pour off excess dye and wash gently in tap water and drain the slide against a paper towel. Expose the smears to Gram's iodine for one minute by washing with iodine, then adding more iodine and leaving it on the smear until the minute is over. Wash with tap water and drain carefully (Do not blot). Wash with 95 percent alcohol for 30. Wash with tap water at the end of the 30 s to stop the decolorization. Drain. Counterstain with 0.25 percentsafranin for 30 s. Wash, drain, blot, and examine under microscope.

Appearance of purple coloured cells indicates negative test that is., sample passes the pathogenicity test. No further testing is required. Appearance of pink or red coloured cells indicates positive test that is, sample fails the pathogenicity test.

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